Molecular Evidence That the Bonin Islands "Honeyeater" Is a White-eye

Mark S. Springer1, Hiroyoshi Higuchi2, Keisuke Ueda3, Jason Minton4 and Charles G. Sibley5

Abstract Since 1958, Apalopteron familiare has been placed in the Meliphagidae and called the Bonin Islands Honeyeater or Ogasawara Honeyeater. Previously it had been treated as a bulb, a babbler, a sylvid warbler and a white-eye. Here we present 12S rRNA sequence evidence that shows that Apalopteron is a member of the white-eye family Zosteropidae, closely-related to the Golden White-eye (Cleptornis marchei) of the southern Mariana Islands, which was also misidentified as a honeyeater until its true affinities were revealed by field observations and DNA-DNA hybridization. The ecology and behavior of Apalopteron and Zosterops are compared and reviewed. The English name Bonin Islands White-eye is proposed for Apalopteron familiare.

Key words: White-eye, Honeyeater, DNA sequences, Zosteropidae, Taxonomy, Ecology.

Introduction

Apalopteron familiare occurs only on the Ogasawara, or Bonin, Islands off southeastern Japan. It was described by Kittlitz (1831) as Ixos familiaris, thus assigned to the bulbuls (Pycnonotidae). Bonaparte (1854) placed it in the monotypic genus Apalopteron and assigned it to the babblers (Sylviidae; Timaliini). Sharpe (1882) restored it to the bulbuls and placed it in Pycnonotus. Delacour (1946) considered it to be a babbler, closely related to Actinodura and Minla.

Deignan (1958) reviewed the tongue structure and other characters, and stated that "It is my contention...that Apalopteron is...a fairly typical genus of the Australasian Meliphagidae or honey-eaters." He added evidence from "pervious nostrils", "quasi-booted tarsi", bill shape, coloration, and "short, somewhat recurved, bristle-like feathers on the front and throat" that seemed to him to be identical to those in species of Meliphaga. The "cup-shaped nest, the materials used, the number of eggs, and the nakedness of the chicks all would, if the bird were native to Australia, be used to confirm the view that it is a typical honey-eater. (It should be mentioned, however, that the eggs of the Australian species of the unspecialized genera seem always to have a pinkish or buffy rather than a greenish blue ground color)."

Deignan's study seemed convincing, but there are no honeyeaters in the Philippines,

Received 29 June 1995, Revised 28 August 1995, Accepted 29 August 1995.
1 Dept. of Biology, University of California, Riverside, CA 92521 USA
2 Laboratory of Wildlife Biology, School of Agriculture and Life Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
3 Dept. of Biology, Rikkyo University, Toshima-ku, Tokyo 171, Japan
4 Wild Bird Society of Japan, Nanpeidai 15-8, Shibuya-ku, Tokyo 150, Japan
5 433 Woodley Place, Santa Rosa, CA 95409 USA
Taiwan or other islands in the northeast Pacific Ocean. Conversely, white-eyes occur on many temperate and tropical islands in the western Pacific, including Japan and nearby islands. Deignan's list of morphological characters includes some that are widely shared among passerines and the tongue structure is not limited to "typical honeyeaters".

Deignan's conclusions were followed by Vaurie (1959). Salomonson (1967: 393) placed Cleptornis marchei and Apalopteron familiare next to one another among the honeyeaters.

The eggs of many species of Zosterops are described as bluish, pale blue, greenish-blue or white, but the eggs of the Golden White-eye (Cleptornis marchei) were described by Hartert (1898: 56) as "pale blue without gloss, spotted over and over with rufous, more so on the thicker end...some...of a deeper sky-blue....The rufous spots are smaller in some, larger and more like blotches in others...." Stinson and Stinson (1994) confirmed Hartert's description of the eggs of Cleptornis marchei. They found 11 nests of which 10 contained two eggs, described as "pale bluish/green with reddish/brown splotches concentrated on the wide end."

Morioka and Sakane (1978) observed the ecology and behavior of Apalopteron and, although they accepted its assignment to the Meliphagidae, considered this relationship "as provisional" (p. 169), noted that Apalopteron and the introduced Zosterops japonica feed on similar foods, and "overlap....in their choice of habitat and coexist...." (p. 178). They discussed tongue structure with reference to Deignan's (1958) study and concluded that "Although we consider Apalopteron to be closer to the honeyeaters than to the babbles in the structure of its tongue, the tongue of certain babbles is not much different and the tongue structure alone of Apalopteron would not be sufficient to distinguish it from the babbles." (p. 180). They noted that the eggs of Apalopteron are "pale greenish-blue, spotted and blotched with brown" (p. 182), thus agreeing with the descriptions of the eggs of the Golden White-eye, noted above. Yamashina (1930: 332–334) also described the eggs of Apalopteron as greenish-blue with brown spots.

The reliance on tongue structure as a taxonomic character has led to erroneous classifications that have included honeyeaters, sunbirds (Nectariniidae, including Prome-rops), flowerpeckers (Dicaeum) and white-eyes in the same higher group, or to place them in sequence in a classification (Sibley and Ahlquist 1990: 612; 652–3; 665–675). Nectar-adapted tongues have evolved independently in other passerines, including leafbirds (Chloropsis), tanagers (Thraupini), Hawaiian honeycreepers (Fringillinae; Drepanidini), wood-swallows (Artamus), wood warblers (Parulini), troupials (Icterini) and some weaverbirds (Ploceinae). In some taxa there is only a tendency toward a nectar-adapted tongue, but it is clear that convergence has produced such tongues in several groups of distantly-related passerines.

Field observations by H. Douglas Pratt on Saipan and Agiguan islands in the southern Mariana Islands caused him to suspect that Cleptornis marchei might be a white-eye, rather than a honeyeater. Pratt et al. (1987: 287) placed Cleptornis marchei in the Zosteropidae and noted that "Until now, this species has been classified as an aberrant honeyeater (Meliphagidae). Pratt's hypothesis, based on behavioral, ecological, and zoogeographical considerations, that the "Golden Honeyeater" is really a white-eye
(Zosteropidae) has been confirmed by biochemical studies in the laboratory of C. G. Sibley (in litt.) at the Yale Peabody Museum. The details of Pratt’s and Sibley’s research will be published in due course. Among the white-eyes, *Cleptornis* appears closest to *Rukia*. Pratt (pers. comm.) believes that *Cleptornis* is closest to *Rukia ruki* of the Truk Group; they have virtually identical songs. Pratt provided a blood sample for DNA extraction and Sibley and Ahlquist (1990: 654) confirmed Pratt’s suspicions with DNA hybridization comparisons. This led Sibley to suspect that *Apalopteron* might also be a white-eye, a suspicion independently shared by Higuchi. Ueda and Higuchi obtained the *Apalopteron* tissues for DNA extraction and Sibley enlisted the collaboration of Mark Springer to perform the DNA sequence study described below. The observations of ecology and behavior are by Higuchi, Ueda, Minton and others whose work is cited.

The DNA hybridization evidence, DNA sequence evidence (below) and similarity of eggshell markings indicate that *Cleptornis* and *Apalopteron* are closely related. If they are treated as congeneric *Apalopteron* Bonaparte 1854 has priority. The generic name *Rukia* was proposed by Momiyama (1922) thus, if the three genera are combined, *Rukia* may be included in *Apalopteron*.

**Materials and Methods**

DNA of *Apalopteron familiare* was extracted from muscle tissue according to the protocol described by Kirsch et al. (1990). DNAs of *Cleptornis marchei* (Golden White-eye), *Zosterops pallidus* (Pale White-eye), *Zosterops lateralis* (Silvereye), *Sylvia curruca* (Lesser Whitethroat), *Melidectes belfordi* (Belford’s Melidectes), *Lichenostomus fuscus* (Fuscous Honeyeater), *Corvus albus* (Pied Crow) and *Tyrannus tyrannus* (Eastern Kingbird) were extracted from blood samples with the procedure described by Sibley and Ahlquist (1990). Approximately 400 base pairs (bp) of the 12S rRNA gene were amplified using the polymerase chain reaction (PCR) (Saiki et al. 1988) with Tfl (Epicentre) and conserved primers designed by Kocher et al. (1989). 35 cycles of amplification were performed, each consisting of denaturation at 94°C for 40 seconds, annealing at 48°C for two minutes and extension at 72°C for three minutes. Amplified sequences were cloned into PCR II (Invitrogen) using one microliter of PCR product and 5.5 ng of vector in each ligation reaction. Ligations were transformed into INVαF’ *Escherichia coli* cells using the procedure of Hanahan (1983) with LB-ampicillin plates. Plasmid DNA was isolated from miniprep cultures using standard techniques (Maniatis, et al. 1982) and sequenced in both directions using the dideoxy chain-termination method (Sanger et al. 1977) with Sequenase 2.0 (United States Biochemical) and 35SdATP. Sequencing primers included vector primers adjacent to the cloning site in PCR II, the Kocher et al. (1989) primers, and the three internal primers B1 (5’ CCTAGAGGAGCCTGTTCCT3’; forward direction), B2 (5’ GACGCGGTATATAGGCT3’; reverse direction), and B3 (5’ AGACAGGTCAAGGTATA3’; forward direction). At least three clones were sequenced for each species and where there was disagreement among clones (always less than 1%) the consensus is reported. Sequences have been deposited in GenBank (Accession Numbers U38350–U38358).
Multiple alignments were made with CLUSTAL (Higgins and Sharp 1988), with minor modifications by eye. Phylogenetic trees were rooted using Tyrannus as the outgroup. Parsimony analyses were performed with PAUP 3.0 s (Swofford 1991) using the branch-and-bound search strategy. Gaps were treated as missing data. The method of Hillis and Huelsenbeck (1992) was used to analyze sequence data for phylogenetic evidence ("phylogenetic signal"). Tests for significantly left-skewed tree-length distributions, which indicate phylogenetic information ("signal"), were made using critical values provided by Hillis and Huelsenbeck (1992). MEGA (Kumar et al. 1993) was used for neighbor-joining (Saitou and Nei 1987) on Kimura (1980)-corrected distances.

Ribosomal RNAs, including 12S rRNA, exhibit secondary structures with unpaired loops and paired stems. In stems, mutations are not independent because of the requirement to maintain base-pairing. Weighting schemes that account for this lack of independence in stems have been developed for nuclear (e.g., Wheeler and Honeycutt 1988; Dixon and Hillis 1993) and mitochondrial rRNAs (Springer et al. in press) in several taxonomic groups. To date, however, there are few complete 12S rRNA sequences for avian species and weighting schemes for mutations in avian 12S rRNA genes have not been developed. Thus, we have not incorporated stem/loop weighting into our analysis.

Results

Multiple Alignment and Nucleotide Differences: Figure 1 shows an alignment of nucleotide sequences for all taxa. The aligned sequences, including gaps, are 401 bases in length. The percentage of sequence divergence ranges from 0.5% (Z. lateralis to Z. pallidus) to 12.6% (Zosterops lateralis to Lichenostomus) among the ingroup taxa. The average distance to the outgroup (Tyrannus) is 16.2%. Transitional differences are more numerous than transversional differences, with a mean transition/transversion ratio of 2.5 for pairwise comparisons between ingroup taxa and for pairwise comparisons between Tyrannus and the ingroups.

Tree Length Distributions: An evaluation of the 135,135 trees for the nine taxa in the study gave a tree-length distribution that is significantly skewed to the left ($g_1 = -0.770$, $P < 0.01$), which indicates that the 12S data contain "phylogenetic signal". Significant phylogenetic signal was also present when only one of the two Zosterops species was included ($g_1 = -0.7242$ for 10,395 trees; $P < 0.01$).

Parsimony Analysis: A branch-and-bound search resulted in a single minimum length tree of 164 steps (see Fig. 2). Fig. 3 shows a bootstrap tree for clades that occur on at least 50% of the 500 bootstrap trees. The following clades occur on the 164-step tree and are also supported at the 80% bootstrap level: (1). Apalopteron and Cleptornis (81%) and these two with Zosterops (95%). (2). Sylvia and the white-eyes, including Apalopteron (90%). (3). Lichenostomus and Melidectes (100%). The honeyeaters associate with the Sylvia/white-eye clade at the 59% level.

Among the nucleotide changes supporting the clades noted above are several unique synapomorphies: (1) Two transitions (C→G at position 61; T→C at position 211) and one transversion (C→G at position 9) in Melidectes and Lichenostomus; (2) a
Fig. 1a.

Fig. 1. Alignment of DNA sequences for part of the 12S rRNA gene.
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**Apalopteron**

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**Lichenostomus**

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**Tyrannus**

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Fig. 1b.
transition (C→T) at position 375 in Apalopteron and Cleptornis; (3) a transversion (C→A) at position 334 in the two species of Zosterops, and (4) one transition (G→A) at position 363) and two transversions (T→G at position 35; G→A at position 363) in Sylvia and the two Zosterops.

**Distance Analysis:** A neighbor-joining tree based on Kimura-corrected distances is shown in Fig. 4. Bootstrap percentages based on 500 replications are also shown. There is high bootstrap support for an association between Cleptornis and Apalopteron (90%), these two with Zosterops (99%) and these three with Sylvia (94%). Melidectes and Lichenostomus cluster together at the 100% bootstrap level and these two are united with the Sylvia-white-eye clade at 60%. These associations are consistent with the parsimony analysis.

**Discussion of the DNA Sequence Data**

Clearly, the 12S rRNA gene contains phylogenetic evidence for the resolution of relationships among these oscine passerines. This is indicated by the left-skewed tree length distributions and the high bootstrap values supporting several clades in the trees.
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Fig. 3. Bootstrap tree for maximum parsimony based on 500 replications. Numbers above branches indicate the percentage of bootstrap support.

The data provide compelling evidence that *Apalopteron* is a zosteropid, not a meliphagid. The close relationship between *Apalopteron* and *Cleptornis* is consistent with their geographic proximity. The 12S sequences also provide strong support for a *Sylvia*-white-eye clade because bootstrap values are approximately 90% for parsimony and neighbor-joining. These results agree with the DNA–DNA hybridization data of Sibley and Ahlquist (1990) which link the Zosteropidae with the Sylviidae, not with the Meliphagidae.

Ecology and Behavior

Japanese White-eyes (*Zosterops japonicus*) are not native to the Ogasawara/Bonin Islands and were not reported by Seebohm (1890) who collected there from May to August, 1889. They were introduced ca. 1900–1910 (Momiyama 1930), and are now common. White-eyes occur on many islands throughout the temperate and tropical western Pacific and native Japanese White-eyes probably occur on all Japanese islands including the Izu Islands and the Volcanic Islands, north and south of the Ogasawara/Bonin Islands. Thus, *Apalopteron* adds the Zosteropidae to the native avifauna of the Ogasawara/Bonin islands. The English name Bonin Islands White-eye is appropriate for this species.

*Apalopteron* shares several ecological and behavioral characteristics with the typical white-eyes (*Zosterops*). Members of a pair or a flock allopreen and roost together in contact on a branch (Higuchi et al. 1993 for *Apalopteron*; Kikkawa 1985 for *Zosterops*).
Bill snapping or beak clattering are used between aggressive individuals (Kikkawa 1985 for Zosterops; Higuchi, unpubl. for Apalopteron). Both sexes participate in nest building, incubation and feeding the young (Higuchi et al. 1993 for Apalopteron; Kikkawa 1985 and Higuchi, pers. observ. for Zosterops). Neither species courtship feeds (Higuchi et al. 1984 for Apalopteron; Kikkawa, pers. comm. and Higuchi, unpubl. for Zosterops). The occurrence of such behaviors tends to be stable within a taxonomic group, although there are exceptions among honeyeaters (R. Noske, pers. comm.) and babblers (C. Yen, pers. comm.) with diverse ecologies and behaviors.
Apalopteron and Zosterops feed on insects, berries and nectar; they overlap almost completely in their choice of habitat and coexist in the same area (Morioka and Sakane 1978). However, they differ in foraging behavior. Apalopteron has longer tarsi and forages among twigs and leaves like white-eyes and tits (Parus), on trunks and branches like nuthatches and woodpeckers and on the ground like small robins (Erithacus) (Higuchi et al. 1995). This differs from the white-eyes which forage in the foliage and small branches of trees and seldom descend to the ground. The diversity in the foraging ecology of Apalopteron is probably the result of niche expansion associated with the absence of tits, nuthatches, woodpeckers and small robins (Higuchi et al. 1995). The morphological differences between Apalopteron and Zosterops may be correlated with the niche expansion of Apalopteron. Similar examples include the Brown Trembler (Cinclocerthia ruficauda) on Dominica Island in the West Indies (Zusi 1969).

The tongues of Apalopteron and Zosterops are illustrated in Fig. 5. Both are cleft at the distal end, but the depth of the split is greater in white-eyes and forms a quadrifid tip. This is correlated with the greater dependence on nectar in Zosterops than in Apalopteron.

Acknowledgments

We thank John Kavanagh and Anthony Metcalf for laboratory assistance. Colleen and Derek Stinson described the eggs and nest of Cleptornis marchei and H. Douglas Pratt provided information about Rukia ruki. Jiro Kikkawa, Richard Noske and Chung-wei Yen helped with information on the ecology and behavior of white-eyes, honeyeaters and babblers, and Hirohiko Sano drew Figure 5. M. S. thanks the Alfred P. Sloan Foundation and the National Science Foundation.

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Molecular Evidence That the Bonin Islands "Honeyeater" Is a White-eye

メグロがメジロ類の1種であることを示す分子生物学的証拠

小笠原諸島の固有種、メグロ Apalopteron familiare は、1958年以降ミツウシ科に分類されているが、これまでにヒヨドリ科、メジロ科、チメジロ科などにも分類されており、分類学上の位置が不安定な鳥である。われわれはミトコンドリア 12S リポソーム RNA をコードする DNA の塩基配列に基づく分子生物学的手法により、メグロがメジロ科の1種であることを示す証拠を得た。また、メジロ科の中では、マリアナ諸島南部に生息するオウゴンメジロ Cleptornis marchei に近縁であることを明らかにした。オウゴンメジロもかつてはミツウシ科とされていたが、最近、DNA-DNA ハイブリダイゼーション法や生態・行動研究などによってメジロ類であることが判明した鳥である。メグロは、相互羽づくろいや接触就眠を行なう、果づくりから育雛まで雌雄で行なう、飼料を供給するなどの生態、行動面でもメジロ類に似ている。また小笠原諸島には元来メジロ類は分布しないことになっているが、メグロがメジロ類の1種になることで分布のこの奇妙な空白も埋まることになる。

マーク・S・シュプリンガー：Dept. of Biology, University of California, Riverside, CA 92521 USA。
楠口広夫：東京大学大学院野生動物学研究室，〒113 文京区弥生1-1-1。
上田恵介：立教大学理学部生物学教室，〒171 豊島区西池袋3-34-1。
ジェイソン・ミントン：日本野鳥の会研究センター，〒150 渋谷区南平台町15-8 ウッディ南平台ビル2F。
チャールズ・G・シャブレイ：433 Woodley Place, Santa Rosa, CA 95409 USA.
Apalopteron familiare on Hahajima Island, the Ogasawara Islands. (Photo; H. Kaneko)